

WHAT IS CLAIMED IS:

- 1 1. A method of creating a library of DNA sequences, said method
2 comprising:
 - 3 a) providing a DNA sequence that encodes a protein of interest;
 - 4 b) providing a probability matrix for the protein;
 - 5 c) providing a constraint vector for the protein;
 - 6 d) applying the constraint vector to the probability matrix to produce a
7 substitution scheme recommending substitutions at at least two residues in the protein;
8 and
 - 9 e) creating a library of DNA sequences incorporating changes in the
10 DNA sequence that produce the recommended substitutions.
- 1 2. The method of claim 1, wherein said protein is selected from the
2 group consisting of an esterase, dehydrogenase and hydrolase.
- 1 3. The method of claim 2, wherein said protein is selected from the
2 group consisting of a protease, cellulase, lipase, hemicellulase, laccase, and amylase.
- 1 4. The method of claim 1, wherein said protein is selected from the
2 group consisting of a transcription factor, growth factor, antibody, interleukin, antigen,
3 and receptor.
- 1 5. The method of claim 1, wherein the probability matrix is based on
2 structural characteristics selected from the group consisting of conservative residues,
3 sequence alignments, three dimensional structure, residue environment, solvent
4 accessibility, residue chemistry, propensity for a particular secondary structure, and
5 combinations thereof.
- 1 6. The method of claim 1, wherein the constraint vector is based on
2 structural characteristics known to affect protein function selected from the group
3 consisting of proximity to the site of functionality, distance of α or β carbons, contact
4 with residues of interest, and contact with residues that contact the residue of interest.

- 1 7. The library of claim 1, wherein said library is a phage library.
- 1 8. A method for screening a library for a protein with an increase in a
2 property of interest, comprising:
- 3 a) providing a probability matrix for a protein of interest;
- 4 b) providing a constraint vector for the protein;
- 5 c) applying the constraint vector to the probability matrix to produce a
6 substitution scheme recommending substitutions at at least two residues in the protein;
7 and
- 8 d) creating a library of DNA sequences incorporating changes in the
9 DNA sequence that produce the recommended substitutions; and
- 10 e) screening the library for a protein with an increase in the property
11 of interest.
- 1 9. The method of claim 8, further comprising identifying a protein
2 having an increase in the property of interest.
- 1 10. A protein produced by the method of claim 9.
- 1 11. A system for creating libraries of nucleic acid sequences that
2 encode variants of a protein, said system comprising:
- 3 a) an initial nucleic acid sequence that encodes a desired protein;
- 4 b) a probability matrix; and
- 5 c) a constraint vector.
- 1 12. A method for improving a desired parameter of a protein of
2 interest, comprising:
- 3 a) providing a probability matrix for the desired protein;
- 4 b) providing a constraint vector for the desired protein;
- 5 c) applying the constraint vector to the probability matrix to produce a
6 substitution scheme recommending substitutions at at least two residues in the protein;
7 and
- 8 d) creating a library of DNA sequences incorporating changes in the

- 9 DNA sequence that produce the recommended substitutions; and
- 10 e) measuring the parameter of interest for at least two members of
11 said library;
- 12 f) determining the sequence for at least two members of said library;
13 and
- 14 g) using sequence comparison and correlation analysis to determine
15 the contribution of mutations or combination of mutations on the parameter measured in
16 step e).
- 1 13. The method of claim 12, wherein the contribution of mutations
2 determined in step g) is used to generate a second library.
- 1 14. The method of claim 1, wherein a library comprising at least 25
2 unique DNA sequences is produced.
- 1 15. The method of claim 14, wherein a library comprising at least 100
2 unique DNA sequences is produced.
- 1 16. The method of claim 15, wherein a library comprising at least 250
2 unique DNA sequences is produced.
- 1 17. The method of claim 16, wherein a library comprising at least 1000
2 unique DNA sequences is produced.
- 1 18. The method of claim 17, wherein a library comprising at least 2500
2 unique DNA sequences is produced.
- 1 19. The method of claim 18, wherein a library comprising at least
2 10,000 unique DNA sequences is produced.
- 1 20. The method of claim 1, wherein a library of less than 10^9 unique
2 DNA sequences is produced.
- 1 21. The method of claim 20, wherein a library of less than 10^6 unique
2 DNA sequences is produced.
- 1 22. The method of claim 21, wherein a library of less than 10^5 unique
2 DNA sequences is produced.
- 1 23. The method of claim 1, wherein the probability matrix is an